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Commentary

Molecular Epidemiology of Hepatitis B Virus Mutants

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INTRODUCTION

Hepatitis B virus is a double-stranded DNA virus that replicates by a process that involves an RNA intermediate and reverse transcription. The viral polymerase has a mutation rate of approximately $<2–10^{-4}$ base substitutions per site per year. The polymerase gene, one of the four overlapping reading frames of the genome, overlaps the S gene, which codes for the surface coat or envelope of the virus. The *a* determinant of the surface antigen spans amino acids (aa) 124–147 between aa 99 and 169, overlapping the major catalytic regions of the viral polymerase protein from aa 454 to 524. Mutations therefore affect the antigenicity of the surface antigen domain. Mutations have also been described in the pre-S genome.

Hepatitis B virus variants with mutations in the polymerase gene of the virus have been described more recently following antiviral therapy with nucleoside analogues, including lamivudine and famciclovir. The mutations are associated with resistance to the respective drugs. It is likely, by analogy to HIV drug therapy, that selection of one mutation by the first drug used will lead to selection of other mutations when the second drug is introduced.

Mutations have also been described in the X reading frame. Hepatitis B *e* antigen results from the translation product of the precore and core regions of the C gene of the virus. It is secreted from the infected liver cell as a consequence of a secretory signal sequence at the precore region. The core antigen is translated from the second ATG of this gene and therefore shares many of the amino acids with the *e* antigen. The protein products contain B- and T-cell epitopes. The *e* antigen may therefore have immunomodulatory functions during the course of infection and may also influence the host cytokine responses. Core/precore mutations are mentioned briefly below.

Precore Variants

In 1978, the occurrence of naturally acquired hepatitis B infection in the colony of chimpanzees of the Zoological Society of London was reported [Zuckerman et al., 1978]. Ten years later the complete nucleotide sequence of the genome of the strain of HBV cloned from the serum of one of the naturally infected chimpanzees was published [Vaudin et al., 1988].

An unexpected feature was a point mutation in the penultimate codon of the precore region changing the tryptophan codon (TGG) to an amber termination codon (TAG). An identical mutation was described subsequently in a number of Greek patients with anti-HBe who also had circulating HBV DNA detected by hybridization [Carman et al., 1989].

Seroconversion of *e* antigen is recognised widely as a sign that active virus replication had declined markedly. However, some patients and carriers develop anti-HBe and continue to replicate HBV at high titre in association precore/core mutants. In precore mutants, synthesis of the nucleocapsid protein is unaltered, but *e* antigen is not synthesised because of a mutation between the two start codons. The *e* antigen is an important target for cell-mediated and antibody-mediated immune responses, so that the loss of *e* antigen production by core mutants may help the virus to evade the host immune response.

At first, it was believed that individuals with core/precore mutants usually progress rapidly to chronic active hepatitis or fulminant hepatitis, but asymptomatic carriers with these mutations have now been detected in most countries [Nakahori, 1995].

Surface Antigen Mutants

The emergence of a variant of hepatitis B virus, possibly due to epidemiological pressure associated with immunisation in an endemic area, was suggested by

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the findings of hepatitis B infection in individuals immunised successfully [Zanetti et al., 1988]. These studies were extended subsequently by the finding of noncomplexed anti-HBs and HBsAg and other markers of hepatitis B infection in 32 of 44 vaccinated subjects, and sequence analysis from one of these cases revealed a mutation in the nucleotide encoding the *a* determinant, the consequence of which was a substitution from glycine to arginine at amino acid position 145 [Carman et al., 1990]. The region in which this mutation occurs is an important virus determinant to which vaccine-induced antibody binds, and the mutant virus is not recognised by antibodies that are capable of neutralising the virus.

A large study in Singapore of 345 infants born to mothers with HBsAg and *e* antigen who received hepatitis B immunoglobulin at birth and plasma-derived hepatitis B vaccine at a dose of 5 μg or 10 μg within 24 hours of birth and then 1 month and 2 months later revealed 41 breakthrough infections with HBV despite the presence of anti-HBs. There was no evidence of infection among 670 immunised children born to mothers with HBsAg and anti-HBe, nor in any of 107 immunised infants born to mothers without HBsAg given the vaccine alone [Oon et al., 1995]. The most frequent variant was a virus in which a single amino acid substitution Gly to Arg occurred at amino acid position 145 of the *a* determinant. Another study in the United States between 1981 and 1993 showed that 94 (8.6%) of 1,092 infants born to carrier mothers became HBsAg-positive after receiving postexposure prophylaxis with hepatitis B immunoglobulin and hepatitis B vaccine. Following amplification of HBV DNA from the *a* determinant region, 22 were found with amino acid substitution (mutation), the majority being in the 142–145 position. 5 had a mixture of wild-type HBV and variants, and the remaining infected children had only the 145 variant [Nainan et al., 1997].

Mutations of HBsAg have been reported from Taiwan, Japan, China, Hong Kong, Thailand, India, Germany, the UK, Brazil, West Africa, and elsewhere. The most frequent mutation reported is the 145 aa variant. Similar mutations have also been described in patients who had undergone orthoptic liver transplantation and treatment with hepatitis B immunoglobulin [reviewed by Oon, 1997; Zuckerman, 1997].

The need for constant epidemiological surveillance is emphasized by the finding in Singapore, between 1990 and 1992, of 0.8% of carriers of HBV variants in a random population survey of 2,001 people [Oon et al., 1996]. There are a number of concerns: there is evidence that HBV surface antigen mutants may not all be detected by all of the current test reagents [Carman and Mimms, 1997; Weinberger et al., 1997] particularly a few based on monoclonal antibodies, and such variants may enter the blood supply or spread by other means, and may infect individuals who develop protective levels of anti-HBs after immunisation. The emergence of these HBV variants may necessitate, therefore, modification of current vaccines to include an antigenic component(s), which will induce antibody to the 145 aa variant. Epidemiological studies remain, therefore, an essential element for the control and prevention of hepatitis B. Mathematical modelling of the impact of large scale vaccination on the prevalence of wild-type HBV and surface antigen variants, assuming an 80% coverage, suggests that virus with the surface antigen mutant(s) will displace the wild-type virus within four or five generations (90–100 years) and that HBV with the surface antigen variants will predominate.

CONCLUSIONS

- Variants of HBV were identified over 10 years ago by researchers in the UK. In 1996, HBV mutants were considered "a mere curiosity and passing fancy in the grand scheme of virus–host interactions" [Baument and Liang, 1996]. However, the more recent identification of surface antigen mutants in the United States [Nainan et al., 1997] has emphasised the potential impact of these observations on the public health.
- There is evidence that HBV surface antigen mutants may not all be detected by all of the current test kits, particularly those kits based on monoclonal antibodies. Such variants may therefore enter the blood supply or spread by other means.
- Current vaccines against hepatitis B do not protect against infection and replication of at the least the predominant 145 aa mutant.
- The 145 aa mutant is replication competent and is stable, and may persist in the host for at least 14 years.
- There is evidence that many of the 10–40% of individuals who have anti-HBc (anticore) antibodies only are low level carriers of hepatitis B virus surface mutants and such carriers may be infectious.
- Epidemiological monitoring of HBV surface mutants is essential employing test reagents which have been validated for detection of the predominant mutations.
- Urgent consideration should be given to the introduction of routine screening by nucleic acid based technology of blood donors and tissue and organ donors.
- Consideration should be given to incorporating into current hepatitis B vaccines of antigenic components which will confer protection against infection by the predominant mutant(s). Such vaccines have been developed on a pilot scale.
- Studies should be undertaken on the molecular epidemiology of drug–resistant mutants.

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